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→ **Immunity –**

The word 'immunity' latin 'Immunis' meaning 'exempt' used to describe the protection against diseases. The power of the body to resist the effects of the invasion of microorganism is called immunity.

Immune system include cells & molecules responsible for immunity of the body & a collective & coordinated response of cells & molecules to foreign substances is k/a as immune response.

Types of immunity :

Immunity

Non-Specific / Innate / Neutral immunity	Specific / Acquired / Adaptive immunity	
– Individual immunity – Racial immunity – Species immunity	Active immunity (AI)	Passive immunity (PI)
	Natural AI	Natural PI
	Artificial AI	Artificial PI

Difference b/w Specific & non-Specific immunity –

Features	Non-specific immunity	Specific immunity
Definition	The resistance to infection that an individual possesses by virtue of genetic & constitutional structure.	The resistance that an individual acquires during life.
Types	Individual, racial & species	Active & passive.

Time taken to develop	Hours	Days
Specificity	For structures shared by groups of related microbes	For antigens of microbes & for non-microbial antigens
Memory	None (Repeated exposure bring i response)	Yes (secondary response much faster than i response)
Physical & chemical barriers	Skin, mucosal epithelia &	lymphocytes in epithelia &
Blood & Tissue Antimicrobial substances	Antimicrobial chemicals Complement, leukins from leukocytes, Plakins from platelets, lactic acid in muscle tissue, lactoperoxidase in milk & interferons	antibodies secreted at epithelial surface Antibodies
Cells	Phagocytes & natural killer cells	Lymphocytes

Difference b/w Active & Passive immunity —

Active immunity	Passive immunity
(1) Developed immunity	Produced immunity
(2) Develops slowly & long-lasting	Relatively fast & short-lived.
(3) A booster dose, if required can be given to give lifelong immunity	A booster dose doesn't help in maintaining it for long.
(4) Prevents a disease & is administered before infection	Develops after the subject has been exposed to an infection
(5) Given in long-term prophylaxis	Given in short-term prophylaxis & therapeutically
(6) Antigens are administered	Antibodies are administered

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Humoral Immunity / Antibody Mediated Immunity (AMI):
The destruction of antigens by producing antibodies is called Antibody mediated immunity & as the antibodies are present in body fluids (humours) is called Humbral immunity.

B-cells cannot bring about humoral immune response but needs the corporation of other cells such as Macrophage (Mf), T-helper cells (T_H), Dendritic cells possess a high levels of surface MHC II molecules that process & present antigens to T-cells.

Antibodies are immunoglobulin (Ig) molecules & they are usually comprised of several categories designated as IgM, IgA, IgE, IgG.

Immunoglobulin participating in HI –
The various Ig participating in HI are as follows:

(1) IgM - It is the first class antibody generated invariably in most humoral responses but normally gets switched over the corresponding IgA, IgE, IgG at very early stage in the immune response.

(2) IgG - Most versatile, important & abundantly available class of antibodies taking part in largest humoral immune reactions. It can cross the placenta, thereby providing a new born baby absolute temporary immunity against the immunogens the mother had earlier against IgG.

(3) IgA - Antibodies are invariably found in tears, saliva, mucous membrane. This immunoglobulin is frequently termed as First-line-of-defence mechanism due to the fact that most bacteria, viruses, fungi that eventually gain entry into the body to cross a mucous membrane.

(4) IgE - It is important in the body's defence against the parasitic worm infection. Prominently & predominantly ied several allergic manifestations give rise to release of erts histamines (eg - allergy due to pollens, house dust, dust mite, human hair, food allergens, etc) which in turn afford the apparent discomforts resulting into extrinsic asthma, hay fever, hives, excessive sneezing (due to seasonal changes & presence of pollen grain in the air).

Mechanism of HMI -
The different types of cells or entities that are held d responsible for contributing to the humoral immunity of are :

(1) B-lymphocytes (B-cells)
(2) Immunodominant peptides (IDPs)
(3) Antigen-Presenting cells (APCss)
(4) T-cell subsets
(5) Class II MHC (major Histocompatibility complex) proteins.

Cell-Mediated / Cellular / T-cell immunity :
Cell-mediated immunity is an immune response that does not involve antibodies or complement but rather al involves the activation of macrophages, natural killer cells, antigen-specific cytotoxic T-lymphocytes & the release of various cytokines in response to an antigen.
Historically, the immune system was separated into two branches :
Cellular immunity for which the protective function of immunisation was associated with cells. CD4 cells or helper T-cells provide protection against different pathogens.

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Humoral immunity for which the protective function of immunisation could be found in the humours (cell-free body fluid or serum).

Mechanism of CMI -
In this type, the body produces large no. of activated lymphocytes that are specially design to destroy foreign organism, hence called cellular or cell-mediated immunity.
It is mediated by T-lymphocytes or T-cells & is also called because of their development & processing occurs in thymus.
T in T-cells stands for Thymus.
B-lymphocytes or B-cells & antibodies have no role play in CMI.

Different cells involved in CMI & their function -

Cell	Function
(i) Helper T cell	Necessary for B-cells activation by T-dependent antigen
(ii) Suppressor T-cell (Ts)	Regulates immune response & helps in maintaining immune tolerance
(iii) Delayed hypersensitivity T-cell (TD)	Provide protection against infectious agent causes inflammation in association to tissue transplant rejection
(iv) Cytotoxic T-cell (Tc)	Destroy target cells on contact
(v) Killer cell (K)	Attacks antibody-coated target cells
(vi) Natural Killer cell (NK)	Attacks & destroy target cells.

"The butterfly counts not months but moments, and has time enough." – Rabindranath Tagor

Importance -
Cellular immunity protects the body are :
(i) Activating antigen-specific Cytotoxic T-lymphocytes that can induce apoptosis in body cells displaying epitopes of foreign antigen on their surface such as virus-infected cells, cells with intracellular bacteria & cancer cells displaying tumour antigen.
(ii) Activating macrophages & natural killer cells, enabling them to destroy intracellular pathogen.
(iii) Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive & innate immune response.

Cellular immunity is directed primarily at microbes that survive in phagocytes & microbes that infect non-phagocytic cells. It is most effective in removing virus infected cells but also participates in defending against fungi, protozoans, cancer & intracellular bacteria.

It plays a major role in transplant rejection.

Note :-

⟹ Immunology -
The branch of medical science that deals with the study of immune response of the body & its interaction with foreign substances including microorganism.
Immunology include study of immunity, immune system & its physiological function, immunological disorder, manipulationing & other physical-chemical as well as physiological character of immune system.

Principle of Immunology :
It is the study of immunity to infection disease in organism include 3 types of protection :

(1) Non-susceptibility - Non-susceptibility characteristic feature of host & vary species to species. It provide a complete protection against a particular foreign particle or microbes.

(2) Natural resistance - The resistance naturally present in the organism & indicate the chemical & physical characteristics of host and vary in different phases of life (age, pathological condition).

(3) Natural immunity - Natural antibodies are responsible for natural immunity & vary person to person & also according to species.

⇒ Difference b/w Antigen & Antibodies -

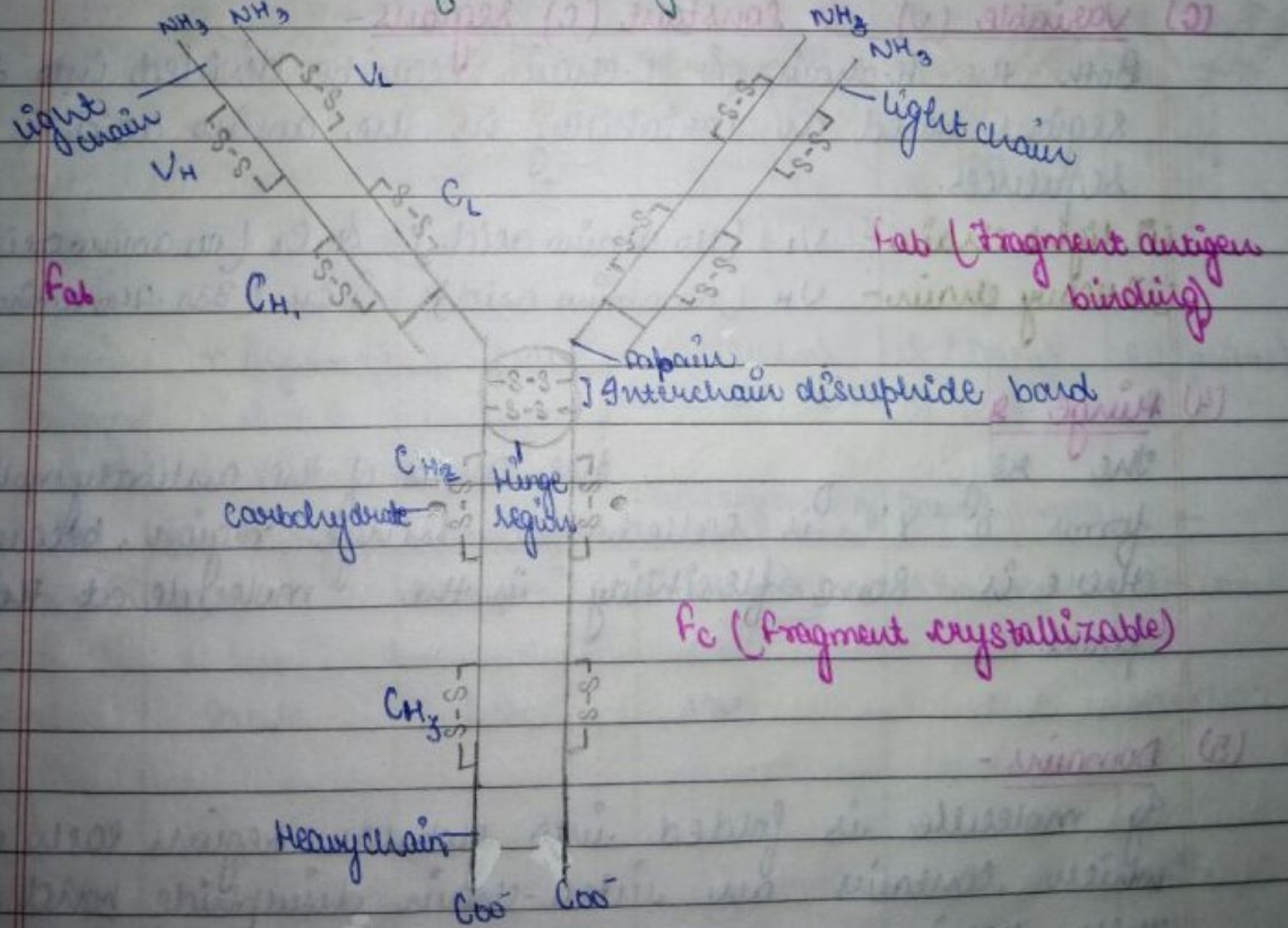
Antigen	Antibodies
• Protein or polysaccharide molecule	Protein molecule
• Foreign material that stimulates antibody formation.	Synthesized by an animal to combat foreign material
• May occur on the surface of a microbe of as a free molecule.	May occur on the surface of a plasma cell & in body fluids
• Binds to a macrophage to reach a helper T-cells to initiate immune response	Directly joins an antigen to destroy the latter.

→ Structure of Immunoglobulins –

Immunoglobulin (Ig) are glycoprotein molecules that are produced by plasma cells in response to an antigen or immunogen & they function as antibodies.

- They are γ globulins.
- They constitute 25-30 % of total serum proteins.
- Antibodies are present in serum, tissue fluids & mucosal surfaces.
- Antibodies are present on the B-cell membrane & secreted by plasma cells.
- All antibodies (Ab) are immunoglobulin but all immunoglobulin may not be antibodies.

Basic Structure of Immunoglobulin :



(a) Heavy & light chains -
All Igs have a four chain structure as their basic unit. One pair of polypeptide chain contains approx twice as many amino acids as the other pair. They are called Heavy (H) chains (50-70 KDa) & light (L) chains (25KDa) respectively.

(b) Disulphide bond -
(i) Interchain: The heavy chain & light chain & the two heavy chain are held together by interchain disulphide bond
(ii) Intra chain: With in each of the polypeptide chains, these are also intra-chain disulphide bonds.

(c) Variable (V) & Constant (C) regions -
Both the H-chain & L-chain can be devided into 2 regions based on variability in the amino acid sequences.
(i) light chain - V_L (110 amino acid) & C_L (110 amino acid)
(ii) Heavy chain - V_H (110 amino acid) & C_H (330-440 amino acid)

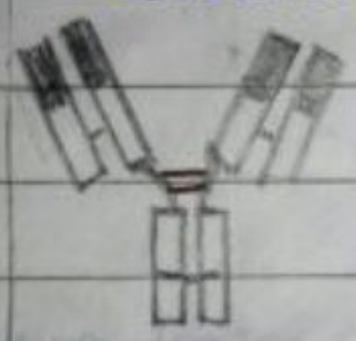
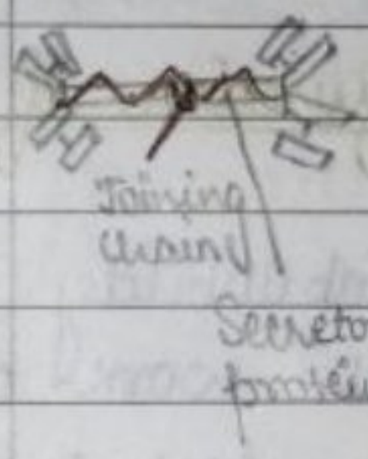
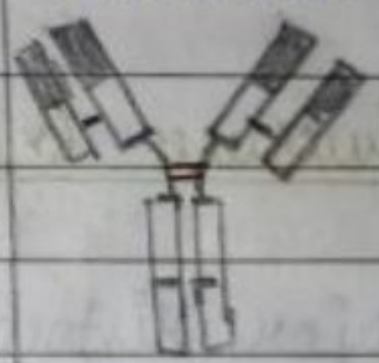
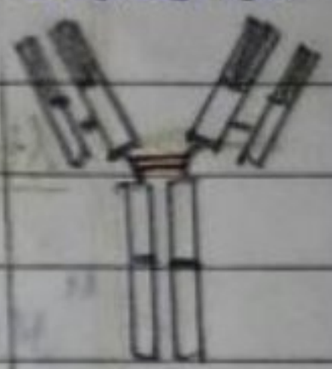
(d) Hinge region -
The region of which the arms of the antibody molecule forms a 'Y' is called the hinge region, because there is some flexibility in the molecule at this point.

(5) Domains -
Ig molecule is folded into globular regions each of which contains an intra-chain disulphide bond. These region are called domains.
(i) light chain domains: V_L & C_L
(ii) Heavy chain domains: V_H & C_{H_1}, C_{H_2}, C_{H_3}

(6) Oligosaccharides :-
Carbohydrate are attached to the C_{H_2} domain in most Ig.

(7) Fragments -
(i) Fab regions : It contain variable section that define the specific target that the antibody can bind.
(ii) Fc regions : All antibodies in a class are the same for each species they are contrast rather than variable.

Types of Immunoglobulin :

Properties	IgG	IgM	IgA	IgD	IgE
Structure	Monomer 	Pentamer 	Dimer 	Monomer 	Monomer 
Heavy chain	γ (gamma)	μ (mu)	α (Alpha)	δ (Delta)	ε (Epsilon)
No. of antigen binding site	2	10	4	2	2
Present in	Serum, lymph	Serum, lymph	Secretory antibody in tear, saliva	Serum, lymphocytes	—
Molecular wt. (Dalton)	150,000	900,000	400,000	180,000	200,000
% of total antibody in serum	80%	6%	13%	<1%	0.002%
Average life in serum (Days)	23	5	6	3	25
Crosses placenta	Yes	No	No	No	No
Fixes Complement	Yes	Yes	No	No	No

Fc bind to	Phagocytes	B-lymphocytes	Phagocytes	B-lymphocytes	Mast cell & Basophils
Present in milk	yes	no	yes	no	no
Function	Neutralization, Agglutination, Complement activation, opsonization & antibody dependent cell-mediated cytotoxicity	Neutralization, Agglutination & complement activation. The monomer form serves as the B-cell receptor	Neutralization & trapping of pathogen in mucus	B-cell receptor	Activation of basophils & mast cells against parasites & allergens

→ Structure & function of MHC –

"Major Histocompatibility complex is a membrane attached protein which work on recognization of antigen b/w self & non-self body & antigen presentation."

MHC molecules always recognize only T-lymphocytes. The two types of MHC are worked in immunity.

Classes of MHC Molecule:

The MHC molecules are classified into 4 classes:

(i) Class I MHC molecules.
(ii) Class II MHC molecules.
(iii) Class III MHC molecules.
(iv) Class IV MHC molecules.

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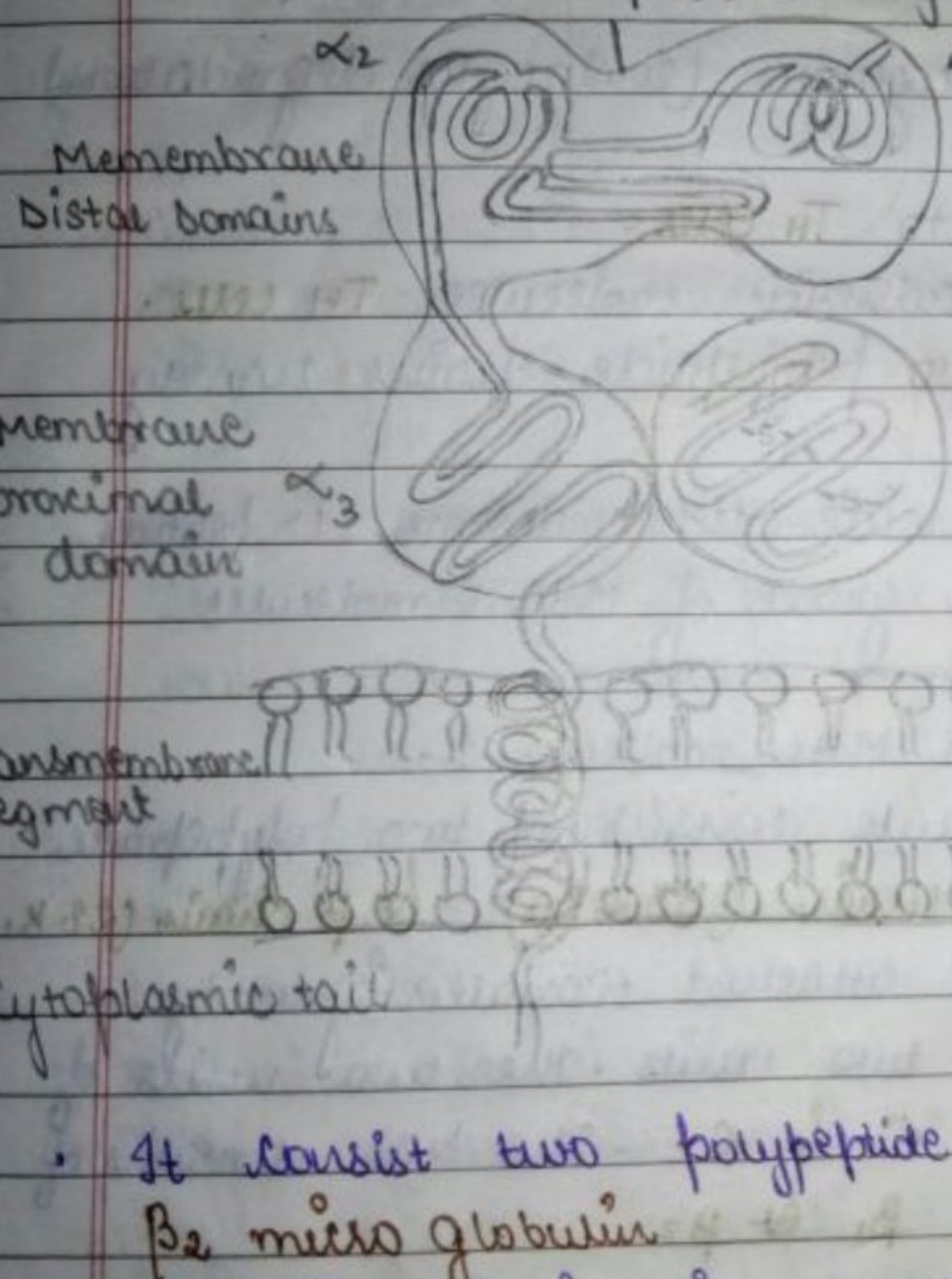
(1) Class I MHC molecules :-

- Class I MHC (45KD) molecule are a group of major histocompatibility antigen.
- They are present on the surface of all nucleated cells except nervous tissue & platelets.
- It present antigen to Tc cells.
- It binds with CD-8 adhesion molecules of Tc cells.
- It bring about cell mediated immune response.

Structure of Class I MHC molecule :-

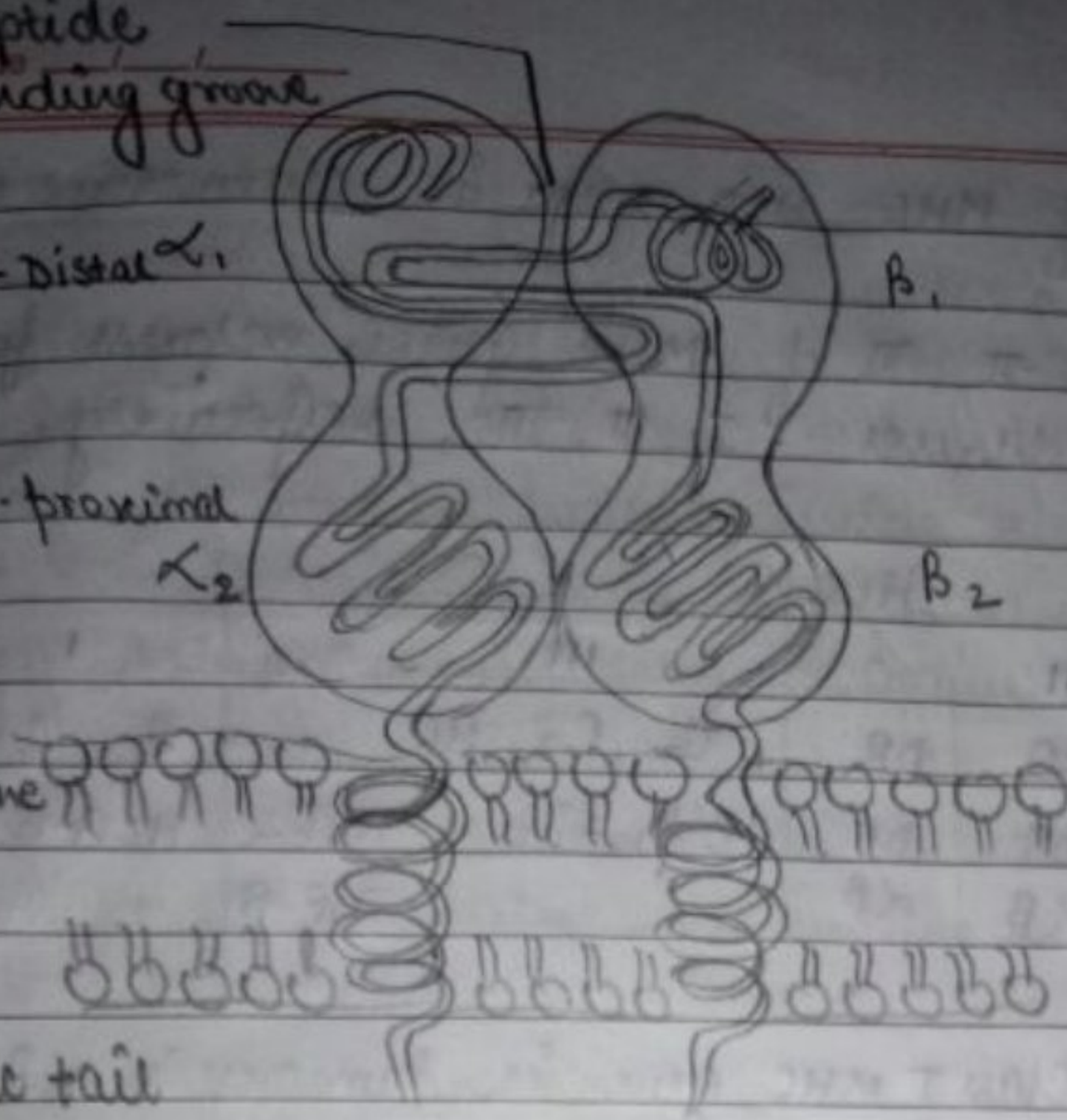


- It consist two polypeptide chains mainly α-chain & β_2 micro globulin
- α chain which is non covalently attached with β_2 microglobulin. α-chain contain a transmembrane glycoprotein which is encoded by A, B, C gene of grouped HLA.
- α-chain is organised by 3 domains such as α_1, α_2, α_3 each domain containing 90 amino acid sequences.
- β_2 microglobulin is similar in size of α_3 & it does not

contain transmembrane proteins.
- When the antigen is internalized & processed inside by proteasome (ubiquitin, cytosolic degradation), the peptides are produced.
- Peptide is further loaded on the groove of MHC I molecules from endoplasmic reticulum.

(2) Class II MHC molecule –
- Class II MHC molecule are present on the surface of antigen presenting cell & cell which engulfed the foreign antigen.
- It binds with the exogenous (endocytic degradation) antigens.
- It present antigen to TH cells.
- It binds with CD4 adhesion molecules TH cells.
- It also consist of two polypeptide chains namely α chain & β chain.
- Antigen is processed inside the endosome & peptide is further loaded on groove of MHC II molecules.

Structure of Class - II MHC molecule :-
- The class II MHC molecule consist of two polypeptide chain namely α chain (33 K.Da) & β chain (28 K.Da).
- The both chain are attached non-covalently.
- Each chain contain two units. The two units of α-chain are called α_1 & α_2. The two domains of β-chain are called β_1 & β_2.
- β_2 & α_2 are transmembrane domains anchoring the same to plasma membrane.
- The α_1 & β_1 domains jointly bear a peptide binding groove.



Class III MHC molecule -
The molecules include components like C2 & C4 & Bf (factor B).

Class IV MHC molecule -
These molecule is present on T cells of leukemia (TLa) as well as on immature thymocytes.

HLA - Human Leukocyte Antigen :
HLA is the MHC molecules present in chromosome 6 in human beings.

- HLA is a set of surface protein present on the surface of all nucleated cells. They are responsible for graft rejection, adaptive immunity, defence against infection, some time it is expressed on cancer cell destruction, Certain autoimmune diseases & Certain Complements.
- MHC is a general term referring to the cell surface antigen of vertebrates.

Organisation of MHC HLA genes in Chromosome 6 in Human

The classes I, II, III of MHC genes express for molecules of classes I, II, III, respectively.

Complex	HLC								
MHC class	II			III		I			
Regions	DP	DQ	DR	C_4, C_2, BF		B	C	A	
Gene Products	DP $\alpha\beta$	DQ $\alpha\beta$	DR $\alpha\beta$	C' proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A	

- The length of Class I MHC gene in humans is ~2,000 Kb (about 20 genes) & is present at the telomeric terminus of the HLA complex.
- The length of class II MHC gene range is ~1,000 Kb & is present at the centromeric terminus of HLA.
- The length of class III genes is ~10,000 Kb & is present between the class I & class II genes.

H-2 complex of Mouse :

- The MHC of mouse is called H2 complex.
- H_2 complex is a cluster of genes responsible for the production of antigen located of nucleated cells & complement components.
- This complex is located in the short arm of the chromosome no. 17.
- It consist of a set of structural genes.
- Haplotypes is a group of linked MHC genes inherited as a unit from parents.

Function :

- MHC molecules are loaded with a bit of sample peptide fragment derived from the degradation of proteins present inside the cell. This peptide is the mirror image of proteins present inside the cells.
- MHC molecules contain self as well as non-self antigen.
- They bring about defense against infection & diseases.
- They mediate certain autoimmune diseases.
- They are responsible for individual smell of people.

→ Hypersensitivity Reaction -

Hypersensitivity (also called hypersensitivity reaction or intolerance) refers to undesirable reaction produced by the normal immune system, including the allergies & autoimmunity. They are usually referred to as an over-reaction of the immune system & these reaction may be damaging, uncomfortable or occassionally fatal. Hypersensitivity reaction require a pre-sensitized (immune) state of the host.

Types -

Gell & Coombs in 1963 classified the identified hypersensitivity responses in four types based on the mechanism of Action.

(1) Type I hypersensitivity - Anaphylactic hypersensitivity
(2) Type II hypersensitivity - Antibody-dependent cytotoxic hypersensitivity
(3) Type III hypersensitivity - Immune-complex mediated hypersensitivity
(4) Type IV hypersensitivity - Cell-mediated hypersensitivity
(5) Type V hypersensitivity - Stimulatory hypersensitivity

Type	Descriptive name	Initiation Time	Antibodies or cell mediators	Immunologic reaction
I	IgE-mediated or Anaphylactic hypersensitivity	2-30 min	Antibody IgE	Antigen (Ag) induces cross linkage of IgE bound to mast cells & basophills along with the release of vasoactive mediators.
II	Antibody-dependent Cytotoxic hypersensitivity	5-8 hrs	Antibody IgM, Antibody IgG, Complement, MAC	Antibody (Ab) directed against cell surface antigens mediate cell destruction by complement activation or ADCC
III	Immune complex-mediated hypersensitivity	2-8 hrs	Antibody IgG, Complement, neutrophills	Ag-Ab complexes deposited in various tissues induce complement activation & an inflammatory response
IV	Cell-mediated or delayed type hypersensitivity	24-72 hrs	T cells	Sensitized TH1 TH2 cells release cytokines that activate macrophages or Tc cells that mediate direct cellular damage
V	Stimulatory hypersensitivity	—	IgM or IgG, Complement	—

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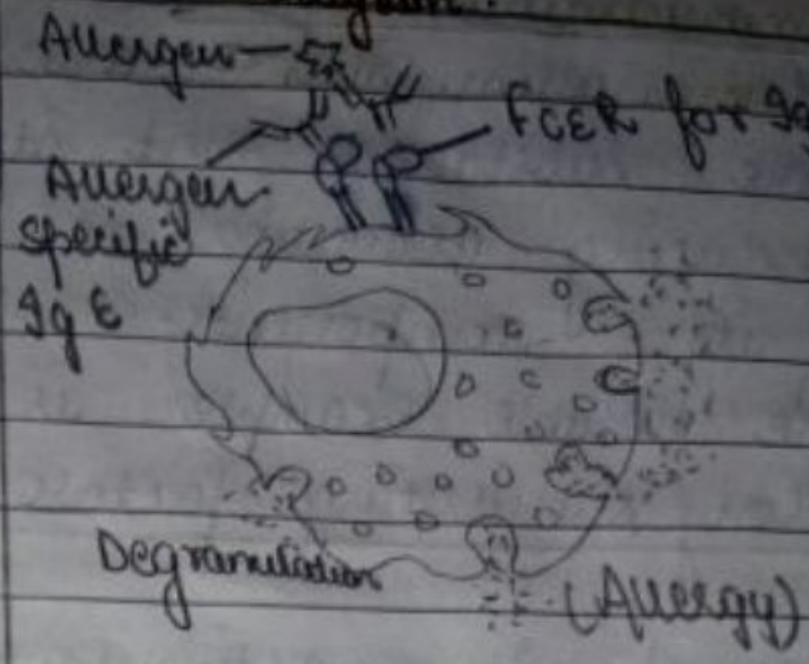
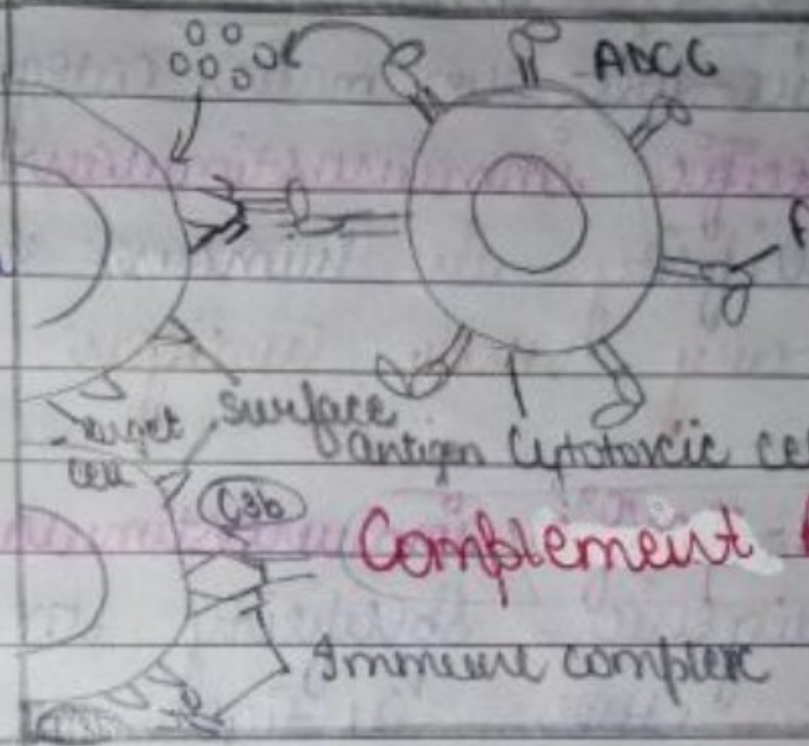
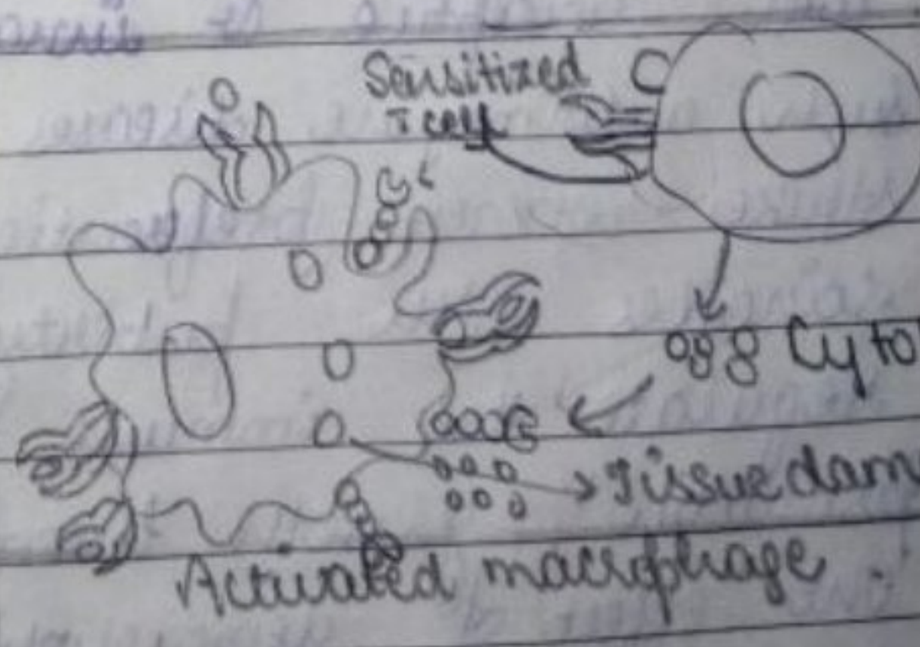
Typical manifestations	Diagram
• Atopy • Anaphylaxis • Hay fever • Asthma • Hives • Food Allergies • Eczema • Churg-Strauss Syndrome	
• Rh-incompatability • Blood transfusion reaction • Autoimmune haemolytic anaemia • Erythroblastosis fetalis • Rheumatic heart disease • Good pasture's disease	
• Serum sickness • Rheumatoid arthritis • Arthus Rx^{n} • lupus nephritis • Post streptococcal glomerulonephritis • Membranous nephropathy • Reactive arthritis • Systemic lupus erythematosus • Extrinsic allergic alveolitis	
• Contact dermatitis • Tuberculoid lesions • Graft rejection • Mantoux test • Chronic transplant rejection • Coeliac disease • Hashimoto's thyroiditis • Granuloma annulare	
• Grave disease • Myasthenia gravis	

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→ Immunostimulation –

Immunostimulants also known as immunostimulators are substances that stimulates the immune system by inducing activation or increasing activity of any of its components.
One notable example is the granulocyte macrophages colony stimulating factor.

Types of Immunostimulants :
There are two main categories of immunostimulants :-

(1) Specific immunostimulants - They provide antigenic specificity in immune response, such as vaccine or any other antigen.

(2) Non-specific immunostimulants - They act irrespective of antigenic specificity to augment immune response of other antigen or stimulate components of the immune system without antigenic specificity, such as adjuvants & non-specific immunostimulatory.

Many endogenous substances are non-specific immunostimulators.
Example: Female sex hormone are known to stimulate both adaptive & innate immune responses.
Such autoimmune diseases such as lupus erythematosus strike women preferentially, & their onset often coincides with puberty. other hormone appear to regulate the immune system as well, most notably prolactin, growth hormone & vitamin D.
The effect of deoxycholic acid (DCA) as an immunostimulant of the non-specific immune system, activating its main actors : the macrophages.
According to these publication, a sufficient amount of

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DCA in the human body corresponds to a good immune reaction of the non-specific immune system.

Some immunostimulating agents :

(1) BCG : In carcinoma bladder
(2) Thalidomide : Different effects of this old drug have been utilised in conditions such as :
(a) Erythema Nodosum leprosum - Anti-inflammatory effect
(b) Multiple Myeloma - Anti-angiogenesis
(c) Rheumatoid Arthritis - Anti TNF effect

(3) Levamisole : An anthelmintic drug that restores functions of B lymphocytes, T lymphocytes, monocytes, macrophages Hence, it has been used in colon cancer along with 5-FU.
(4) Recombinant cytokines :
(a) Interferons : In tumours & chronic hepatitis B & C
(b) Interleukin 2 (Aldesleukin) - In renal cell carcinoma & melanoma.

→ Immunosuppressants -

- Immunosuppressant drugs are a class of drugs that suppress or reduce the strength of the body's immune system. They are also called anti-rejection drugs.
- One of the primary uses of immunosuppressants drugs is to lowers the body's ability to reject a transplanted organ such as lives, Heart or kidney.
- Almost everyone who receives an organ transplant has to take immunosuppressant drugs. The body recognizes a transplanted organ as a foreign mass. This triggers a response by the body's immune system to attack it.
- By weakening the immune system, immunosuppressant drugs

decreases the body's reaction to the foreign organ. The drugs allow the transplanted organ to remain healthy & free from damage.

- Immunosuppressant drugs also are used to treat autoimmune diseases, such as lupus. An autoimmune disorder is a disease process in which the body attacks its own tissue. Lupus results from just such a misdirected activity of the body's own immune system. By suppressing this reaction, immunosuppressant drugs can help control the impact of the disease on the body.

- Other diseases treated with immunosuppresant drugs include:
  - → Psoriasis
  - → Rheumatoid arthritis
  - → Crohn's disease, a chronic inflammation of the digestive tract.
  - → Alopecia areata (Patchy hair loss)

Classification of Immunosuppressants:

(1) Selective Inhibitors of Cytokine production & function - Cyclosporine, Sirolimus, Tacrolimus (FK506)

(2) Antibodies - Alemtuzumab, Daclizumab, Anti-Thymocyte globulins (ATG).

(3) Immuno suppressive Antimetabolites - Azathioprine, Mycophenolate mofetil

(4) Adrenocorticoids - Methylprednisolone, Prednisolone, Prednisone

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Side effects of Immunosuppressants :

- nausea, vomiting, diarrhoea or stomach ulcers.
- Rash
- kidney inflammation.

Rare side effects -

- Fever
- Inflammation of the pancreas.
- Suppression of Blood cell prodⁿ (Bone marrow suppression) which may increase the risk of infection or serious bleeding. Return to normal blood cell production may take several weeks after the medicine is stopped.

→ Immunological Products -

The term 'Vaccine' has been derived from the latin word 'vacc' which means 'cow'.

Acc. to medical definition, "Vaccines are pharmaceutical suspension or sol" of immunogenic substances intended to induce active immunity".

"Vaccination is the administration of a vaccine to help the immune system develop protection from a disease."

Classification :

- Diagnostic agent
  - Milk test toxin
  - Old tuberculin
  - Tuberculin PPD
- Immune Blood derivatives
  - Human normal immunoglobulin
  - Boiled human normal immunoglobulin

Immunological Product

For Active immunisation

- **Bacterial vaccines**
  - BCG vaccine
  - Cholera vaccine
  - Pertussis vaccine
  - Typhoid vaccine
- **Viral & Rickettsial vaccine**
  - Smallpox vaccine
  - Yellowfever vaccine
  - Measles vaccine (live)
  - Poliomyelitis vaccine (oral)
  - Rabies vaccine
  - Typhus vaccine
- **Prep^{n} Containing Toxoids**
  - Diphtheria toxoids
  - Tetanus toxoids
  - Diphtheria & tetanus vaccine (adsorbed)
  - Diphtheria, tetanus, pertussis vaccine (adsorbed)

For Passive Immunisation

- **Antitoxin**
  - Diphtheria antitoxin
  - Tetanus antitoxin
  - Gas-gangrene antitoxin
- **Antiviral Serum**
  - Rabies antiserum
- **Antibacterial Serum**
- **Immune Blood derivaties**
  - Human normal immunoglobulin
  - Dried human normal immunoglobulin

As diagnotic agent

- Schick test toxin
- old tuberculin
- Tuberculin PPD

Classification:

"Vaccination is the administration of a vaccine to help the immune system develop protection from a disease."

Method of the Preparation :

(A) Active Immunisation :-

(1) Bacterial Vaccine -

Bacterial vaccine are either Sterile suspension of live or killed bacteria or Sterile extracts of derivatives of bacteria.

They may be simple vaccines prepared from one species or may be mixed vaccines prepared by mixing two or more simple vaccines from different species or varieties.

Bacterial vaccines may be prepared from cultures grown on suitable solid or liquid media. The bacteria are then suspended in normal saline sol^{n} or freeze dried.

Vaccine containing living bacteria may be prepared from strains which are avirulent for man but which can stimulate the prod^{n} of antibodies active against pathogenic strains of the same species.

The bacterial vaccine must be free from any substance known to cause toxic, allergic or other undesirable immunological reaction in man. eg - BCG vaccine.

Vaccine containing killed organisms may be prepared by killing the organism by chemical or physical means provided the allergic potency of the vaccine is preserved. eg - Cholera vaccine, Pertussis vaccine & Typhoid vaccine.

→ Bacillus Calmette - Guerin or BCG Vaccine :-

BCG vaccine is in the form of white pellet which when reconstituted yields an opalescent suspension. It is a freeze - dried prep^{n} containing live culture of the Bacillus of Calmette & Guerin strain of

Mycobacterium tuberculosis var. bovis.

Preparation -
The bacilli are grown on a suitable culture media resulting when plated out on suitable solid culture media, should not less than 20 million colonies. The growth period should not be more than 14 days in any case.
After a suitable growth, they are separated by filteration in the form of a cake. The cake is homogenised in a grinding flask & suspended in a suitable sterile liquid medium designed to preserve the antigenicity & the viability of the vaccine. The suspension is transferred into the final sterile container & freeze dried. The containers are sealed so as to prevent contamination or deterioration of the vaccine. The vaccine contain no antimicrobial agent.

Storage and Stability -
Store in hermetically sealed light resistant glass containers at a temperature between 2° - 8°C. It is more stable if stored at temperature as low as -20°C. It should not be frozen.

Use -
BCG vaccine is used as an immunising agent which provides protection against tuberculosis.

Dose -
Prophylactic 0.1 ml as a single dose by intracutaneous injection

Labelling -
The label state that -
(i) the no. of viable particles.

(iii) The name & volume of the liquid to be used for reconstituting the vaccine.
(iii) The storage condition.
(iv) The expiry date.
(v) That any portion of the reconstituted vaccine should not be exposed to light before or after re-constitution.

→ **Cholera vaccine:-**

Cholera is colourless, whitish or slightly brownish opalescent liquid, free from clumps & containing not less than 12,000 million bacteria in 1 ml. The pH should be between 6.8 - 7.4.
It is a sterile homogenous suspension of killed cholera vibrions (vibrio cholera) of strains selected for high antigenic efficiency & purity.

Preparation -
Equal portion of the strain Inaba & ogawa of vibrio cholera are used of preparing cholera vaccine. These strains are selected to provide high proficiency against antigens. Initially, each strain of vibrio cholerae is grown individually on a solid medium for 1-2 days (seed-lot system). Then the bacteria are washed with a normal saline sol^{n}. Thereafter, the bacterial suspension is either heated at 56°C temperature for 1 hour or treated with a bactericide (eg- phenyl or formaldehyde). The vaccine can be added with a preservative. This process kills the suspended bacteria.

Storage & Stability -
Cholera vaccine should be stored at 2-8°C temperature & should not be frozen.

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Use -
. It is used for immunisation against cholera.
. The vaccine doesnot prevent transmission of the uts
disease after the limited period of 3 to 6 months.

Dose -
Prophylactic, initial dose 0.5 ml, second dose 1 ml after an interval of 4 to 6 weeks.

Labelling -
The label on the container should state -
(i) the no. of bacteria / ml
(iii) Storage conditions
(iii) "not to be frozen".
(iv) The date after which it is not be used.
(v) "shake well before use".
(vi) The name & proportion of any added preservative.

→ Pertussis vaccine (whooping cough vaccine) :-
It is available as more or less turbid, whitish liquid nearly odourless or having faint odour due to antimicrobial agent.
Pertussis vaccine is a sterile bacterial suspension of killed pertussis bacilli (Bordetella pertussis) of a strain or strains selected for high antigenic efficiency.

Preparation -
It is prepared by culturing Bordetella pertussis in a suitable culture media. It is separated, washed & suspended in normal saline sol^n. The bacteria are killed either by heating or by adding some chemicals. The suspension is standardised. The vaccine may show abnormal toxicity in animal tests

& this is removed by cold storage for upto 3 months.

Storage & Stability -
Store at a temperature b/w 2° to 8°C & should not be frozen.

Use -
For active immunization of children against whooping cough especially when diphtheria and tetanus toxoids & pertussis vaccine (DPT) causes untoward reaction or is contraindicated.

Doses -
It is administered by S.C. injection in three doses of 0.5 ml, 1.0 ml, 1.5 ml at least 4 weeks apart. (2)

→ Typhoid vaccine :-
Typhoid vaccine is white or creamy, white turbid liquid free from clamps.
It is a sterile suspension prepared from one or more strain of Salmonella typhi that are smooth & have the full components of O, H & Vi antigens.

Preparation -
Salmonella typhi organisms are grown on a suitable culture media. The bacteria are killed by heat or by a bactericide such as phenol, formaldehyde or by a chemical such as acetone. It is then standardised so that 1.0 ml of the typhoid vaccine contain not less than 1000 million bacteria (S. typhi). The vaccine must comply with tests for sterility & the test for undue toxicity for vaccine.

Storage and stability -
Store at a temperature b/w 2°-8°C. The vaccine should be frozen.

Use -
It is used for immunization against infection caused by typhoid bacilli.

Dose -
Prophylactic, initial dose 0.5 ml followed by second dose of 1.0 ml by S.C. injection after an interval of 4 to 6 weeks.

(2) Viral vaccine and Rickettsial vaccine -
Viral & rickettsial vaccines are suspension of viruses or rickettsial. They are prepared from infected tissue of blood obtained from artificially infected animals, from cultures in fertile eggs or from cell of tissue cultures.
Viral vaccines may be live or killed.
* live vaccines are usually prepared using attenuated strains of specific organism.
* Killed vaccines may be inactivated by suitable chemical or physical means.
These vaccines may be freeze dried.

→ Smallpox vaccine :-
Smallpox vaccine is almost white powder which reconstitutes to yield a viscid, straw coloured liquid.
Smallpox vaccine contains living attenuated vaccinia virus.

Preparation -
Smallpox vaccine is a freeze-dried suspension of the living virus of vaccinia, of a strain capable of protecting man against smallpox. The virus is grown in the skin of healthy animals, usually young cattle or sheep. The product is suspended in a sol having stabilising properties & then distributed into its final sterile containers & freeze-dried. It may contain a suitable preservative.

Reconstituted vaccine complies with the requirements of identification, test for living contaminants, undue toxicity, potency & stability. The requirement for sterility does not apply to this vaccine.

Storage & Stability -
Store at a temp. b/w 2°-8°C, when the reconstituted vaccine retains its potency for 7 days. The stability of the vaccine can be tested by keeping the freeze-dried vaccine at a temp. b/w 35°-37°C for not less than 4 weeks after which the potency is not less than 1/10th of the originally found.

Use -
Immunising agent.

Dose -
For prophylaxis of smallpox, about 0.02 ml applied to the skin & inoculated by scarification or pressure

Labelling -
The label on the container states -
(i) The directions for reconstituting the vaccine.

(ii) The vaccine is to be administered into the skin by scarification & is not to be injected.
(iii) The animal in which the vaccine was prepared.
(iv) The date of expiry which should not exceed 6 months from the date on which the test for potency was carried out.

→ Yellow fever vaccine :-
It is a white, slightly yellow or light brown scale or powder. Yellow-fever is an aqueous suspension of chick embryo tissue infected with the 17D strain of yellow fever virus.

Preparation -
The virus is injected into the embryo of fertile eggs which have been incubated for 7-8 days. After incubation for further 3 to 4 days, the embryos are removed, pooled in batches, ground & then extracted with purified water. The suspension is then centrifuged. The supernatant liquid is separated to which a suitable preservative may be added. It is then distributed into sterile glass ampoules & freeze-dried. The air is removed from the ampoules or replaced by oxygen-free nitrogen before these are sealed.

Storage & Stability -
Yellow fever vaccine is stored in the dark at a temp. of approx. 0°C. The vaccine loses its potency within a few days if it is stored at a high temp.

Use -
It stimulate production of antibodies against yellow fever

Dose -
Prophylactic, by S.C. injection not less than 1000 LD50 doses.

→ Measles Vaccine live :-
The vaccine is white or nearly white friable mass
(i) Measle vaccine live is a bacteriolly sterile aqueous suspension of a suitable live, attenuated strain of measles virus grown on culture of chick embryo cells.

Preparation -
The strain of attenuated measles virus used in the manufacture of live vaccine is tested on the monkeys for freedom from neuro vaccine. The strain is grown with aseptic precaution in cultures of chick embryo cells. The chick embryoes are derived from healthy & pathogen free flocks. Only 1° cell culture are used in the manufacture of the vaccine. The growth of virus is done with in a 14 days of inoculation. The virus suspension is tested for identify, Sterility & for being free from adventitious viral agents. The cultures which pass these tests are pooled & clarified to remove intact tissue cells. A suitable stabilizer is added & it is distributed into sterile containers, freeze dried & sealed. The vaccine must comply with the test for sterility & also the test for undue toxicity.

Storage & Stability -
The vaccine is stored in a single dose in light resistant container at a temp. b/w 2°-8°C The reconstituted vaccine should be used immediately after prep^n.

"The butterfly counts not months but moments, and has time enough." – Rabindranath Tagore

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Use -
It produces active immunity against measles in appsox. 99%. of recipients of a single dose. its

Dose -
Paediatric, by S.C injection, 0.5ml of reconstituted vaccine.

→ Poliomyelitis vaccine (oral) :-
It is clear liquid which may have a reddish colour if phenol red has been used in the prep^n.
Poliomyelitis vaccine (oral) is an aqueous suspension of one or a combination of 2 or 3 types of live attenuated strains of poliomyelitis virus tested for neuro-virulence on monkeys. It is a polyvalent vaccine.

Preparation -
It is prepared by using three strains of poliomyelitis virus Type I, II, III. The virus of each type is grown separately in cultures of suitable tissue, with aseptic precautian. The tissue should be free from extraneous micro-organism & adventitious agents. Suitable antibiotic other than penicillin & streptomycin may be used in small conc^n. The medium is maintained at a temperature not exceeding 35°C during the growth of the virus.
The growth of the virus is done within 4 days of inoculation & the virus suspension is tested for identity, sterility & freedom from adventious viral agents. The final vaccine is prepared by combining the appropriate dilution of three virus types & by the addition of approved bactericide. The vaccine must comply with the tests for sterility &

the test for undue toxicity for vaccine.

Storage & Stability -

This is potent if stored at temperature below -20°C until the expiry date indicated on the vial. It can be stored for six months at 5 ± 3°C temperature.

→ Hybridoma Technology or Monoclonal Antibodies -

Monoclonal antibody can be defined as a type of antibody derived from hybridoma cells.

Monoclonal antibodies (MAbs) are homogenous, immunological reagents of defined specificity, so that these can be utilized for diagnosis & screening with ease & certainity.

Hybridoma Technology :

Hybridoma Technology is a method for producing large no. of identical antibodies (also called monoclonal antibodies) The myeloma cells line that is used in the process is selected for its ability to grow in tissue culture & for an absence of antibody synthesis.

History :

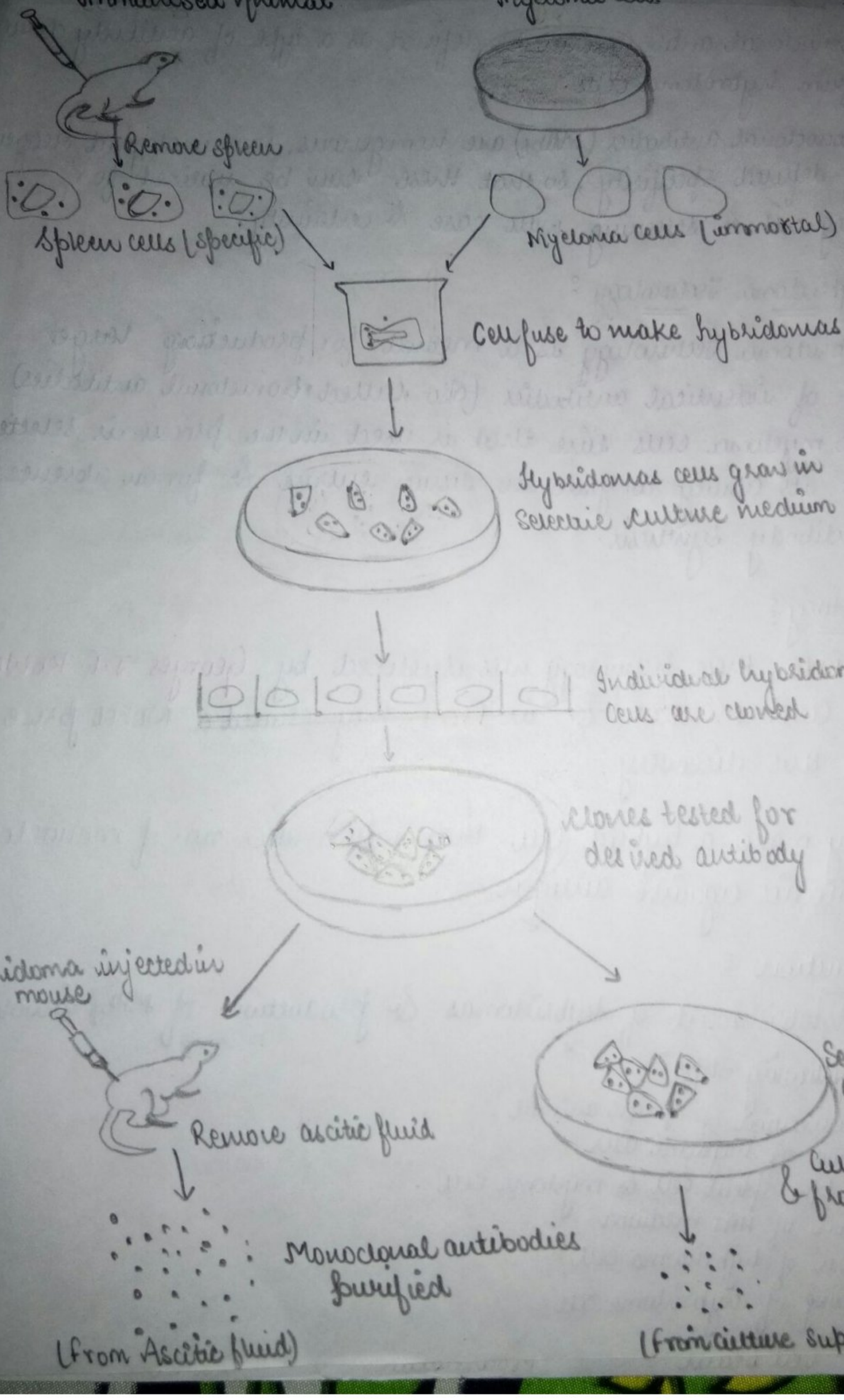
- In 1975, these technology was developed by Georges J.F. Kohler & Cesar Milstein. & in 1984, they shared a Nobel prize for this discovery.
- They make a hybrid cell that will make no. of monoclonal antibodies against antigen.

Production :

The establishment of hybridomas & production of MAbs involves the following steps:

(a) Immunisation of specific animal.
(b) Isolation of myeloma cells.
(c) Fusion b/w spleen cell & myeloma cell.
(d) Selection of HAT medium.
(e) Isolation of hybridoma cell.
(f) Screening of hybridoma cell.

Production of MAbs by Hybridoma Technology



(1) Immunization of specific animal -

An antigen immunized to an animal (like mice) via intravenously (directly to blood) by injection.

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where in spleen it activate B-cell which produce plasma cell (spleen cell).

↓

Plasma cell to produce monoclonal antibodies.

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Isolation of plasma cell from spleen of animal.

(2) Isolation of Myeloma cells.

- Myeloma cells are cancerous cells which isolated from bone marrow.
- Myeloma cells are generally immortal in nature (that never dies) & has multiplication property.

(3) Fusion of spleen cell & Myeloma cells.

- It requires PEG (Poly ethylene glycone) medium for fusion.
- It can also done by electrofusion.
- Fusion b/w spleen cell & myeloma cell produced five different types of cells:

(a) Fused Plasma
(b) fused myeloma
(c) Hybridoma
(d) unfused plasma
(e) unfused myeloma

(4) Selection of HAT medium - (Hypoxanthine, Aminopterin, Thymidine)

- Before multiplication of Antibody, it has to synthesize new copy of DNA & for that it requires synthesis of nucleotide.
- for synthesis of nucleotide mainly 2 pathways are there -

(1) Salvage pathway (2) De-novo synthesis

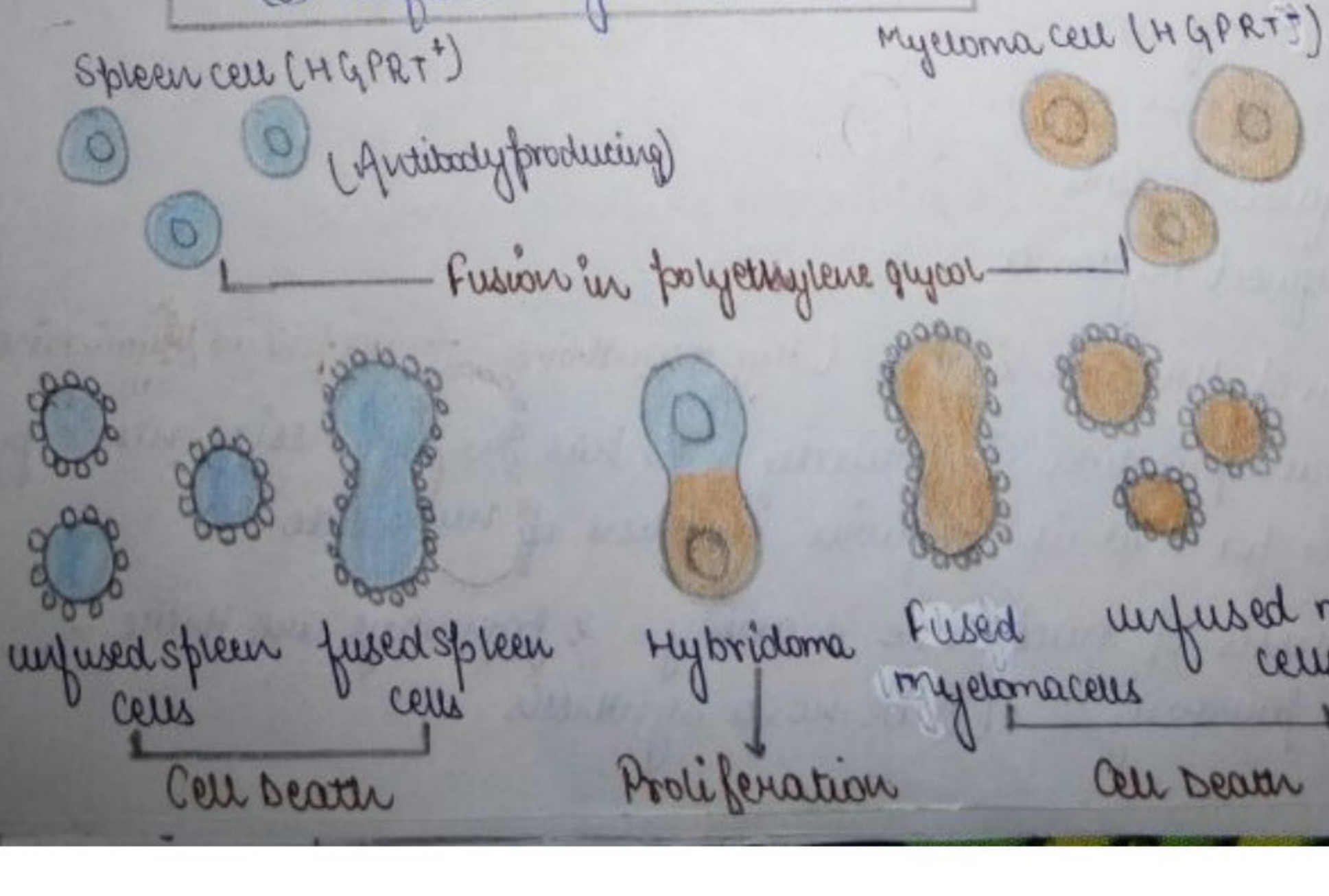
- In salvage pathway, it requires degraded part of old nucleotide to produce new nucleotide.
- In De-novo synthesis, it synthesized completely new nucleotide by small molecules (sugar, amino acid)
- So in HAT medium, cells not synthesized by De-novo synthesis due to presence of Aminopterin in HAT medium which blocks Di-hydro Folate Enzyme which is necessary for these synthesis.
- For synthesis in Salvage pathway it must require Hypoxanthine Guanine Phospho-Ribosyl Transferase (HGPRT) enzyme.
- Where hypoxanthine & thymidine are used as precursors.

(5) Isolation of hybridoma cell -

Spleen cell have HGPRT enzyme

Myeloma cell doesn't have HGPRT enzyme

	HGPRT
(1) Fused plasma	Present
(2) Fused myeloma	Absent
(3) Hybridoma	Present
(4) Unfused plasma	Present
(5) Unfused myeloma	Absent



- Fused myeloma & unfused myeloma didn't have HGPRT enzyme so can't survive in HAT medium.
- Fused plasma & unfused plasma have HGPRT enzyme but didn't have long life.
- Hybrid cell has HGPRT enzyme from spleen cell as well as they have the ability to multiply repeatedly as myeloma cells.
- So, isolation of hybrid cell because is only cell which survive in HAT medium.

(6) Screening of hybridoma cell -

- Elisa screening method which done by incubating hybridoma culture in which secondary enzyme gets conjugate & formation of colored product shows positive hybridoma.
- Used for multiplying the hybridoma cells :

(1) In-vivo

(2) In-vitro

- In vivo procedure, involves introduction of hybridoma cells into the peritoneal cavity of the animal then from ascetic fluid antibodies are isolated.
- In vitro method involves culturing of hybridoma cells in suitable culture media & then antibodies are isolated & purified.
- Once a hybridoma colony is established, it will continuously grow in culture medium like RPMI-1640 & produce antibodies.
- Storage : liquid Nitrogen.

Purification of Antibodies:

- MAbs may need to be purified before they are used for a variety of purposes.
- Antibodies can be purified by anyone of the following techniques:
  (a) ion-exchange chromatography.
  (b) antigen affinity chromatography.

Serum Free Media for Bulk Culture of Hybridoma Cells –

- The use of serum, however, leads to difficulties in purification of antibodies.
- It is an expensive technology for large scale production of hybridoma cells for industrial production of monoclonal antibodies.
- In view of these difficulties, serum free media are being ↑singly used for culturing hybridoma cells.

Advantages of Serum free Media in hybridoma cell culture & prep^{n} of monoclonal antibodies:

(1) Greatly simplified purification of antibodies due to increased initial purity & absence of contaminating immunoglobulin.
(2) Decreased variability of culture medium.
(3) Reduced risk of infectious agent.
(4) Fewer variables for quality control / quality assurance.
(5) Increased control over bioreactor secretion.
(6) Potential for increased antibody secretion.
(7) low or no dependence on animals.
(8) cost effective.
(9) overall enhanced efficiency.

Disadvantages of Serum free media in Hybridoma cell culture & prep^{n} of monoclonal antibodies :

(i) Not all Serum free media are applicable to all cell lines.
(ii) Cells may not grow to as high densities & may be more fragile than cells in Serum.
(iii) Media may take longer to prepare.

Uses of Monoclonal Antibodies :

(a) Monoclonal Antibodies or Specific antibodies are now essential tool of much biomedical research & are of great commercial & medical value.
(i) Diagnosis (including ELISA test for detection of viruses & imaging)
(ii) Immunopurification
(iii) Therapy.

(b) MAbs are used for instance to distinguish subsets of B cell & T cells.
(c) In diagnosis, pregnancy can be detected by assaying of hormones with monoclonal.
(d) Monoclonal antibodies are being used to track cancer antigens & alone or linked to anticancer agents, to attack cancer metastases.
(e) The MAbs are known as OKT$_3$ is saving organ transplants threatened with rejection & preventing bone marrow transplants from setting off graft-versus-host disease (immune system series).

Application of Hybridoma Technology :

(i) Serological - Identification of ABO blood group.
(ii) Diagnosis -
- Detection of pregnancy by assaying of hormones with monoclonal.
- Separation of one substance from a mixture of very similar molecules.

(3) Immunopurification –

- Purification of individual interferon using monoclonal.
- Inactivation of T-lymphocytes responsible for rejection of organ transplants.

(4) Therapy-

- Removal of tumor cell from bone marrow.
- Treatment of acute renal failure.
- Treatment of malignant leukemic cells, B cell lymphomas & a variety of allograft rejection after transplantation